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TO	Examiner D. Crouch Art Unit 1632	FAX	703-872-9306
FROM	Charlton Shen CS	PAGES	6 (INCLUDING THIS SHEET)
PHONE		DATE	2/2/2004
RE	Directed Differentiation of Embryonic Cells		
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COMMENTS

Please see attached

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Benvenisty, N.	Atty Dkt:	1822/113
Serial No:	09/918,702	Art Unit:	1632
Date Filed:	July 31, 2001	Examiner:	Crouch, D.
Invention:	**Directed Differentiation of Embryonic Cells**	Date:	February 2, 2004

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence is being filed via facsimile transmission to: Examiner Deborah Crouch, Ph.D., Art Unit 1632, of the United States Patent and Trademark Office, 703-872-9306 on February 2, 2004.

Charlton Shen

Commissioner for Patents
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COMMENTS REGARDING ADVISORY ACTION OF 30 DECEMBER 2003

Dear Madam:

The Applicant's representative thanks the Examiner for the opportunity to clarify the Applicant's position regarding the Advisory Action mailed on 30 December 2003 during a telephone interview on 8 January 2004. In accord with 37 C.F.R. § 1.133(b), the following comments summarize the interview and provide augmentation of the subject matter discussed.

Pending claims 8 - 16, which all require "permitting a population of human embryonic stem cells to form embryoid bodies" and "causing directed differentiation of said embryonic cells," are not rendered obvious in light of combining Keller (Curr. Opin. in Cell Biology, Vol. 7, pp. 862-869 (1995)) and Thomson et al. (Science, Vol. 282, pp. 1145-1147 (1998)) because there is no reasonable expectation that using the methods of Keller on human embryonic stem (ES) cells would have been successful.

The Advisory Action of 30 December 2003 maintains that Keller in combination with Thomson renders pending claims obvious despite the arguments provided in the Applicant's response of 14 November 2003. In summary, the Action maintains that Reubinoff et al. (2000) (reference AW in the IDS submitted with the regular application on January 31, 2001) does not show that there was no reasonable expectation of success in applying Keller's methods to human embryonic stem cells ("hES cells"). A review of Reubinoff and the application, however, clearly indicates that at the time the application was filed, there was no reasonable expectation that Keller's methods would allow the formation of embryoid bodies in hES cells, a required step of the pending claims.

A. Reubinoff, and other references, Show That Embyroid Body Formation Techniques for Murine ES Cells Fail when Applied to Human and Primate ES Cells

Keller reveals methods for forming embryoid bodies from *murine* ES cells. In particular, Keller notes that embryoid bodies may be formed from murine ES cells that are initially maintained in a pluripotent state on embryonic fibroblasts, or in the presence of leukemia inhibitory factor ("LIF"). See Keller at 862. The embryoid bodies may be formed by (a) removing the murine ES cells from the presence of the feeder cells or LIF; (b) by a "hanging drop" method; or (c) placing the murine ES cells in the presence of stromal cells. See Keller at 861-862.

Reubinoff clearly indicates that such techniques would be expected to fail when applied to human ES cells. In particular, Reubinoff grew hES cells on a feeder layer. See Reubinoff section entitled **"Differentiation of human ES cells in vitro."** The reference notes specifically that "[c]ultivation of clumps of ES cells in *hanging-drop cultures, or as aggregates on bacteriological petri dishes*, in standard medium without feeder cells resulted in considerable cell death." See id. (emphasis added). Thus, Reubinoff tried two of the methods discussed by Keller on hES cells and the methods killed the hES cells. As well, when the hES cells were cultivated to high density on a feeder layer "there was no consistent pattern

of structural organization suggestive of the formation of embryoid bodies similar to those formed in mouse ES cell aggregates." Thus, Reubinoff shows another technique that forms embryoid bodies in mouse ES cells, but does not form embryoid bodies with human ES cells. These results are consistent with the findings that embryoid body formation techniques for murine ES cells also fail in marmosets and rhesus monkeys. See Applicant's Response C at 12. Thus the prior art at the time of filing of the application clearly shows no reasonable expectation of success in forming embryoid bodies with human ES cells, a necessary step of all the pending claims. Thus, Keller and Thomson do not render the pending claims obvious.

B. The Embryoid Body Formation Techniques of the Application Differ From the Techniques of Keller

As discussed during the telephone conference, the application supports the required claim element "permitting a population of human embryonic stem cells to form embryoid bodies" in a manner that differs from simply applying one of the embryoid body formation techniques of Keller to human ES cells. Indeed, Thomson notes that human ES cells act similarly whether in the presence or absence of LIF. See Thomson at 1146, column 1. This is in contradistinction to the Keller teaching that murine ES cells will form embryoid bodies when removed from LIF, but maintain an undifferentiated, pluripotent status when the murine ES cells are in the presence of LIF. See Keller at 862. Thus the protocol for embryoid body formation of hES cells taught in an embodiment of the invention described in the application differs from the techniques shown in Keller. See Application at 14 – 15. For example, one protocol described indicates that "ES cells were transferred using trypsin/EDTA" before embryoid bodies are formed from the hES cells. See id. at 14. Such a step is not mentioned in Keller before embryoid body formation takes place with murine ES cells.

C. **Responses to Advisory Action**

The Action states that Reubinoff does not suggest that the failure to form embryoid bodies affects the response of exogenous factors. The pending claims are method claims that require the step of "form[ing] embyroid bodies." Thus if Keller and Thomson cannot teach the step of embryoid body formation with hES cells, the combination cannot render the pending method claims obvious.

As well, the application notes that embryoid body formation "allows complex signaling to occur between the cells," and thus is an important step in achieving directed differentiation. See Application at 8, lines 26 – 29. Indeed, the claims are not drawn to a method causing any amount of differentiation of hES cells, but *directed* differentiation. See Application at line 32, page 8 through line 6, page 10 (describing growth factor embodiments of the invention for *directed* differentiation, and showing examples where the differentiation is not simply random).

The Action also suggests that since Keller teaches other methods of forming embryoid bodies with murine ES cells beyond what is mentioned by Reubinoff, an obviousness rejection may be maintained. The references presented by the Applicant show, however, that no embryoid body formation technique successful in murine ES cells was successful when applied to human ES cells. Indeed, the *reasonable* expectation is that murine techniques do not work with hES cells at the time of the application's filing. It cannot be argued that it would have been *reasonable* to assume that stromal cells could form embryoid bodies with hES cells when all other techniques were shown to have failed (including 2 of the 3 mentioned in Keller). As stated in MPEP § 2143.03, "at least some degree of predictability is required" for an obviousness rejection. Given the failures shown in the references, no degree of predictability exists. Indeed, assuming that the stromal cell technique would be successful is not only impermissible hindsight but even more unsupported since no showing in the application, or cited in an office action, exists to indicate that the stromal cell

technique would be successful in forming embryoid bodies from hES cells as part of a method of directed differentiation at the time of filing of the application.

In no way can Keller's techniques as applied to human embryonic stem cells render the pending claims obvious, given the teachings of Reubinoff and other references.

The Applicant's representative cordially requests the allowance of pending claims 8 – 16.

Respectfully submitted,

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